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Research Article

Trichoderma asperellum, a potential fungal biocontrol agent against *Aspergillus niger*

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Abstract: *Trichoderma asperellum* is free-living, ubiquitous fungus which is very common in the habitat of soil and root ecosystem, is known as a potent opportunistic, avirulent plant symbionts and it parasitizes several soilborne phytopathogens. *Aspergillus niger* is well known black mold which causes several storage diseases. Among the storage diseases, black mould disease of onion is an important disease which is caused by *A. niger*. Antagonsitic potential of *T. asperellum* was assayed against three isolates of *A. niger* [RC1, RC2 (isolated from soil samples of Farm 1 and 2) and RC3 isolated from diseases onion]. Antagonistic efficacy of *T. asperellum* of *A. niger* almost similar against all the test isolates. Percentage inhibition of radial growth (PIRG) of *A. niger* by *T. asperellum* inhibited 55.17% within five days, 77.20% within 7 days and 92.06% in 12 days. Antagonistic efficacy of *T. asperellum* can be exploited in the management of black mould disease of onion.

Keywords: *Trichoderma asperellum, Aspergillus niger,* Black mould, Antagonistic potential, Inhibition.

1. Introduction

Onion (*Allium cepa*) is one of the most important vegetable cum condiment crops which belongs to family Alliaceae. In India, Onion is widely cultivated under three cropping seasons, i.e., Kharif, Late Kharif and Rabi. It has valued for their medicinal and therapeutic properties as well as has numerous health benefits likewise treating sun strokes, prevention of cancer and cardiovascular disorders [1]. Besides of several medicinal properties, the onion plants has also affected by insects, pest and diseases during the cropping season as well as in storage too. Among the diseases, a black mould disease on onion is caused by *Aspergillus niger* is a major postharvest disease-causing pathogen as well as it also reduces the marketable quality [2]. The disease-causing pathogen survives in soil as a saprophyte which infects onion rootlets or neck in the field and finally establishes in bulb of onion [3]. Hayden and Maude [4] reported that 30 to 80% spoilage of onion bulbs during storage is caused by *A. niger*.

Biological control of plant pathogens by microbial bioagents has been considered a more natural and environmentally acceptable alternative to the chemical/synthetic fungicides [5]. Among the beneficial microorganisms, *Trichoderma* species are highly exploited fungal bioagents throughout the world for the management of soilborne diseases, plant growth promotion and inducing systemic resistance in crop plants to enhancing their resistance level [6]. Several reports indicated that the *Trichoderma* species have the ability to antagonize a wide range of soilborne phytopathogenic fungi viz., *Sclerotium rolfsii*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, *Macrophomina phaseolina*, *Fusarium* species [6,7,8,9]. *Trichoderma* species have competed with soilborne phytopathogens for nutrients and space, direct antagonisms, secreting antibiotics as well as inducing systemic resistance of plants as well as stimulate plant growth promotion by producing of plant growth promoting molecules [10,11]. Several reports indicated that the antagonistic potential of *Trichoderma* species highly depended on the lytic enzymes, *e.g.* chitinases and β-1,3-glucanases [12,13]. Occurrence of *Trichoderma* spp., a very common inhabitant in the organic-rich soil, but their population density drastically reduced due to intensive farming without using organic manures and excessive use of synthetic fertilizer and pesticides in Jharkhand [8].

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The overall aim of this work was to expand the knowledge of using biocontrol potential of bioagents as well as to find out suitable fungal biocontrol agents for the management of storage loss of onion bulbs. The specific objectives of this work to exploits native biocontrol microbes for the management of *A. niger*, a black mould of onion causing phytopathogen.

2. Materials and Methods

2.1 Isolation and maintenance of *T. asperellum* and *A. niger*

The study was conducted during 2013-2014 at the ICAR-Research Complex for Eastern Region, Research Centre (ICAR-RCER RC), Plandu, Ranchi, Jharkhand. The isolate of *T. asperellum* NAIMCC-F-03167 was obtained from the unit of Plant Pathology, ICAR-RCER RC, Ranchi while three isolates of *A. niger* viz., RC1, RC2 from soil and RC3 from diseased onion were isolated and culture were maintained on PDA medium (peeled Potato 200g, Dextrose 20gm, Agar 15-18gm and distilled water 1 L) under aseptic conditions for keeping the culture fresh and viable. Pure cultures of *A. niger* were identified based on the basis of colony morphology, microscopic characters were matched with manual and monograph of *Aspergillus* [14,15].

2.2 Antagonistic efficacy of *T. asperellum* against three isolates of *A. niger*

Antagonistic efficacy of *T. asperellum* against three isolates of *A. niger* were studied under strict aseptic laboratory condition using dual culture plate techniques [16]. Fungal mycelium of the *T. asperellum* (5-7 days) and *A. niger* isolates (RC1, RC2 and RC3) cut separately with the help of sterilized cork bores (5mm disc). A disc of *A. niger* isolates were placed on the periphery of solidified PDA in Petri plates, (3.5cm approx.) in triangle and disc of *T. asperellum* was placed in the centre of triangle and experiments were conducted in triplicate. Inoculated Petri plates were incubated at 27 ± 2^0C in BOD and data's were recorded periodically after 5, 7 and 12 days interval. Inhibition of radial growth (in mm) of *A. niger* were measured after 5, 7 and 12 days of inoculation.

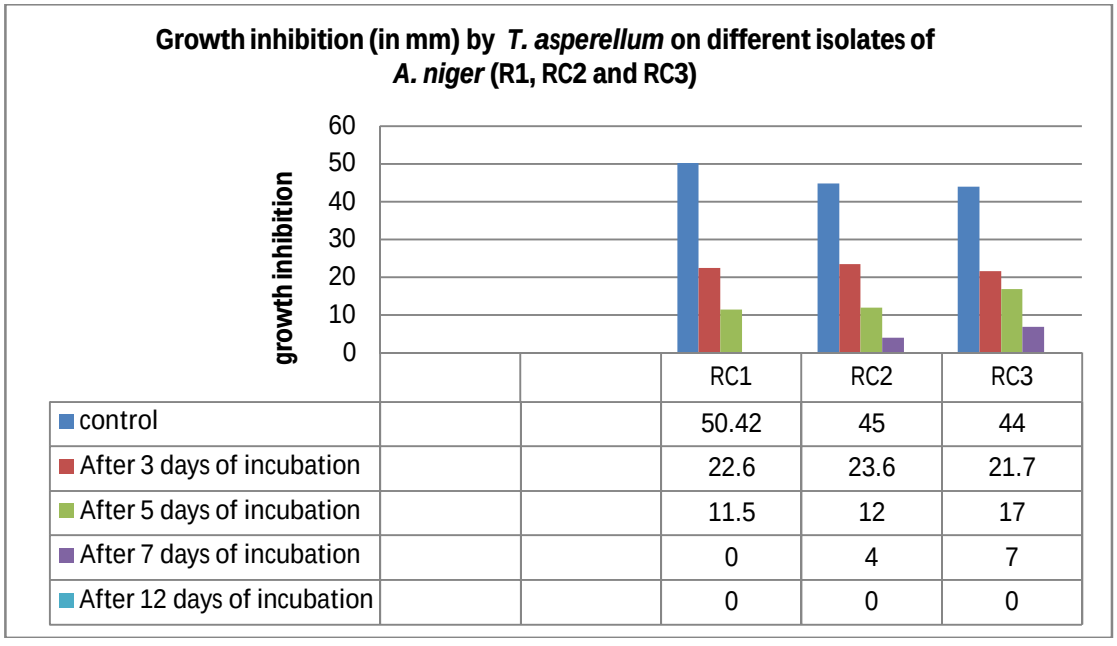
Percent inhibition of *A. niger* was calculated using the following formula [17]:

$$I = \frac{C-T}{C} X\ 100 \quad (1)$$

Where, I = Percent inhibition;
C = Radial growth in Control;
T = Radial growth in the treatment.

3. Results and discussion

T. atroviride, *T. harzianum*, *T. koningii*, *T. viride* and *T. pseudokoningii* have been reported as the best antagonists for white rot disease of onion, caused by *Sclerotium cepivorum* [18,19]. Antagonistic efficacy of *T. asperellum* against three isolates of *A. niger* showed highly encouraging results within span of five days of inoculation which reduced >40% mycelial growth while 77.20% in seven (7) days of inoculation. *T. asperellum* completely eliminated within 12 days of inoculation (Fig. 1) against all the three isolates of *A. niger*.



Graph 1. Antagonistic activity of *Trichoderma asperellum* on different isolates of *A. niger* is showing 100% growth inhibition after 12 days of incubation.

T. asperellum also proved to be effective in containing the growth of *A. niger* samples, which were isolated from the soil sample (RC1, RC2 and RC3). It limited the impact of *A. niger* by reducing the radial growth of *A. niger* by 53.19% within three days of their interaction and by 76.19% within 7 days and containment by 92.06% by the passage of 12 days (Fig. 1) in RC1 plate. The antagonistic impact of *T. asperellum* on *A. niger*, in other used, replicates remained almost the same as proved by repeating the same *in vitro* experiment as reflected in (Fig. 1, Graph 1). The effort to contain the growth of *A. niger*, isolated from another soil sample (RC1) received a boost as *T. asperellum* yielded nearly the same result. *T. asperellum* limited the impact of *A. niger* by reducing the radial growth of *A. niger* by 58.01% within three days of their interaction and containment by 86.11% by the passage of 7th day and 100% control/elimination within 12 days. The antagonistic impact of *T. asperellum* on *A. niger* in inhibiting its radial growth, in other used replicates, remained almost same as confirmed through repeating the same *in vitro* experiment (Fig. 1, Graph 1). Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the means of the four variables at the 95.0% confidence level (Table 1).

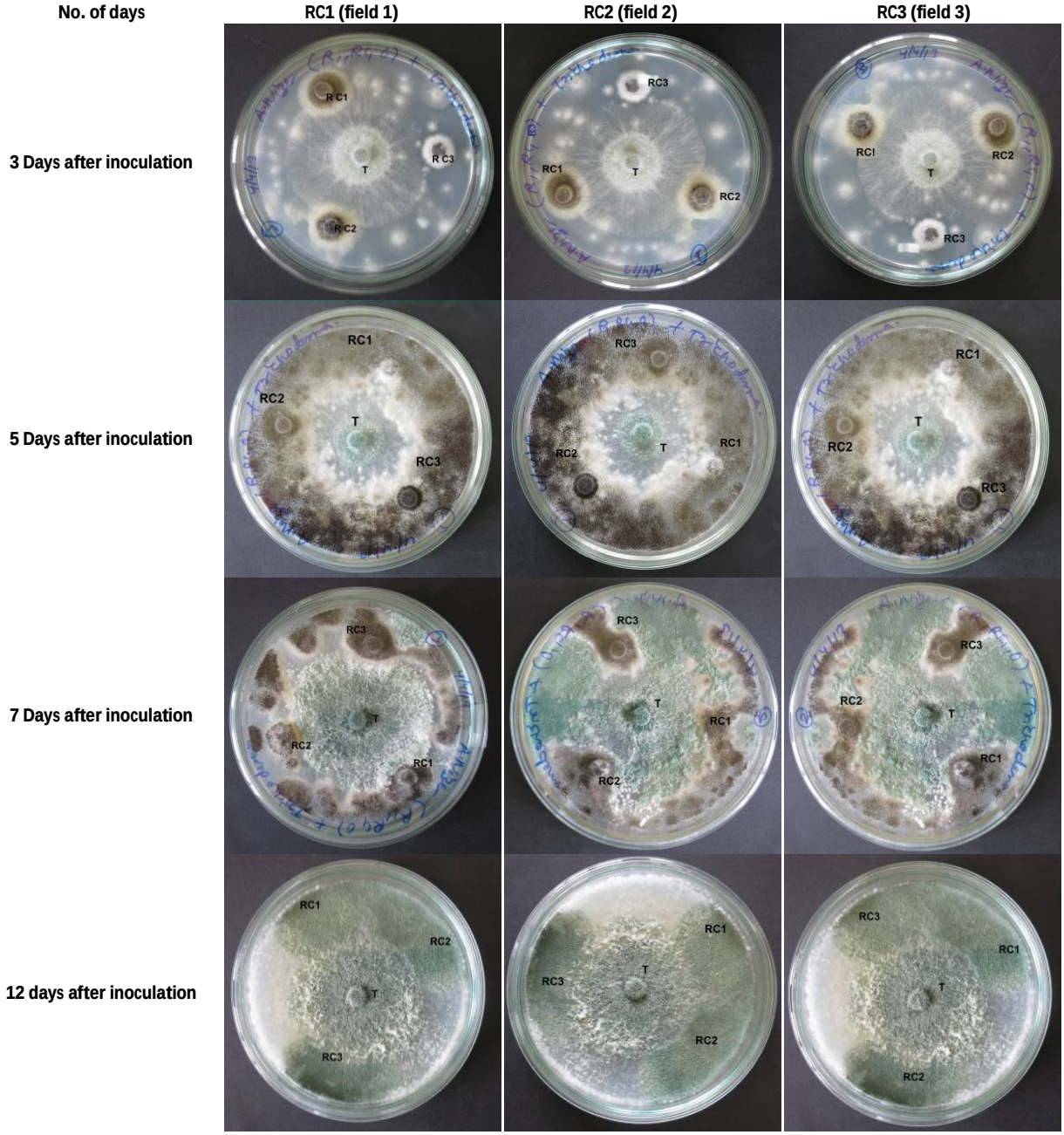


Fig. 1. Antagonistic activity of *Trichoderma asperellum* against different isolates of *A. niger* by dual culture method, after 3, 5, and 7 days of inoculation antagonist overgrowing on pathogen after 12 days of inoculation 100% growth inhibition.

Table 1. Statistical analysis (ANOVA) by Statgraphic Software.

No. of days	Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
After 3 days of inoculation	Between groups	1190.07	3	396.691	97.33	0
	Within groups	32.6043	8	4.07553		
	Total (Corr.)	1222.68	11			
After 5 days of inoculation	Between groups	2574.72	3	858.241	186.25	0
	Within groups	36.8643	8	4.60803		
	Total (Corr.)	2611.59	11			
After 7 days of inoculation	Between groups	2574.72	3	858.241	186.25	0
	Within groups	36.8643	8	4.60803		
	Total (Corr.)	2611.59	11			
After 12 days of inoculation	Between groups	4311.61	3	1437.2	387.16	0
	Within groups	29.6976	8	3.7122		
	Total (Corr.)	4341.31	11			

4. Conclusion

Antagonistic efficacy of *Trichoderma* species against *A. niger* revealed that the isolates of *T. asperellum* to have highly effective against all the tested isolates of *A. niger* which inhibited their radial growth by 52 to 58% just within 5 days of their interaction. Moreover, 86% to 92% reduction of radial growth in *A. niger* after 12 days of interaction were recorded. As per results, it can be concluded that antagonistic impact of *T. asperellum* can be exploited as an eco-friendly effective tool in control storage pathogens.

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